

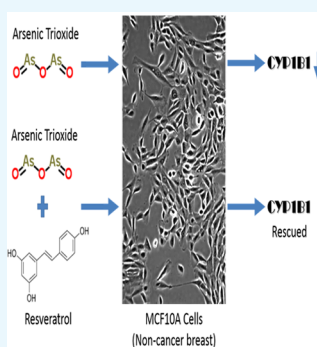
Modulation of Cellular Response to Arsenic Trioxide Toxicity by Resveratrol

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Supporting Information

ABSTRACT: Arsenic trioxide (As_2O_3) is an environmental carcinogen and a putative endocrine disruptor. Resveratrol has been shown to reverse As_2O_3 -induced oxidative damage. In immortalized but nontransformed estrogen receptor α -negative human breast cells (MCF10A), we observed that 25 μM resveratrol ameliorated As_2O_3 -induced cytotoxicity. As_2O_3 , in the presence or absence of 25 μM resveratrol, induced quinone reductase (NAD(P)H quinone dehydrogenase 1), via the induction of NFE2-related factor 2. As_2O_3 caused a repression of cytochrome P450 (CYP)1B1, but the addition of 25 μM resveratrol rescued the expression of cytochrome P450 1B1 and kept it at a constant level. Therefore, 25 μM resveratrol can modulate the effects of As_2O_3 on enzymes involved in estrogen metabolism.



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INTRODUCTION

Arsenic is the 20th most abundant element in the earth's crust, and trivalent arsenic trioxide (As_2O_3) contaminates groundwater in many places, leading to arsenic poisoning or arsenicosis.¹ Arsenic and inorganic arsenic compounds have been classified as IARC Group 1 carcinogens,² with lung, bladder, kidney, and liver as the predominant targets. At the same time, As_2O_3 (Trisenox or arsenic trioxide) is being used as a chemotherapy drug against acute promyelocytic leukemia (APL), where it selectively kills the leukemic cells but allows the proper development of regular blood cells.³ Interestingly, arsenic has long been implicated in endocrine disruption. Target genes for glucocorticoids, androgens, mineralocorticoids, and progesterin receptors have also been reported to be regulated by inorganic arsenic in a biphasic dose-response fashion.⁴ To address such documented carcinogenicity and putative endocrine disruption, the U.S. Environmental Protection Agency has set the maximum contaminant level for As_2O_3 at 10 ppb.⁵

The principal pathways implicated in As_2O_3 toxicity result from reactive oxygen species, oxidative DNA damage, and induction of apoptosis. Resveratrol, a well-known dietary stilbene, has been shown to protect normal human bronchial epithelial cells from As_2O_3 toxicity by maintaining glutathione homeostasis.⁶ Cardiotoxicity, a major side effect of using As_2O_3 for APL, could be ameliorated in Wistar rats by resveratrol, via the maintenance of a balanced expression of the NFE2-related factor 2 (Nrf2)-heme oxygenase (HO) 1 pathway, and by promoting arsenic efflux from cells.⁷ Employing similar

mechanisms, resveratrol has been reported to protect from As_2O_3 -induced nephrotoxicity in male Wistar rats⁸ and from hepatotoxicity in Chinese Dragon-Li cats.⁹ Finally, research by at least one group has demonstrated the ability of inorganic arsenic to promote carcinogenesis via a nonestrogen receptor (ER)-mediated pathway: chronic exposure (18 weeks) to environmentally relevant 0.5 μM arsenite promoted cancer cell phenotypes in human prostate epithelial stem/progenitor cells (WPE-stem)¹⁰ and in their isogenic parental RWPE-1 cells (30 weeks).¹¹ Chronic exposure (24 weeks) to low-level arsenite (500 nM) has also been reported to do the same thing in human breast epithelial cells (MCF10A) via the overexpression of aromatase.¹² To the best of our knowledge, no studies have explored any protective role of resveratrol in As_2O_3 -induced carcinogenesis via non-ER-mediated pathways.

Estrogens have been implicated in the development of a variety of cancers. Several types of evidence suggest the role of estrogen in tumor development in an ER knock-out transgenic mouse model of breast cancer,¹³ as well as in the transformation of ER α -negative breast epithelial cells (MCF10F).¹⁴ To explain such receptor-independent pathways for cancer initiation, it has been hypothesized that the metabolism of estrogens/androgens generates catechol quinones that can react with DNA, leading to the formation of apurinic sites and mutations, to initiate oncogenic transformation.¹⁵ In high-risk

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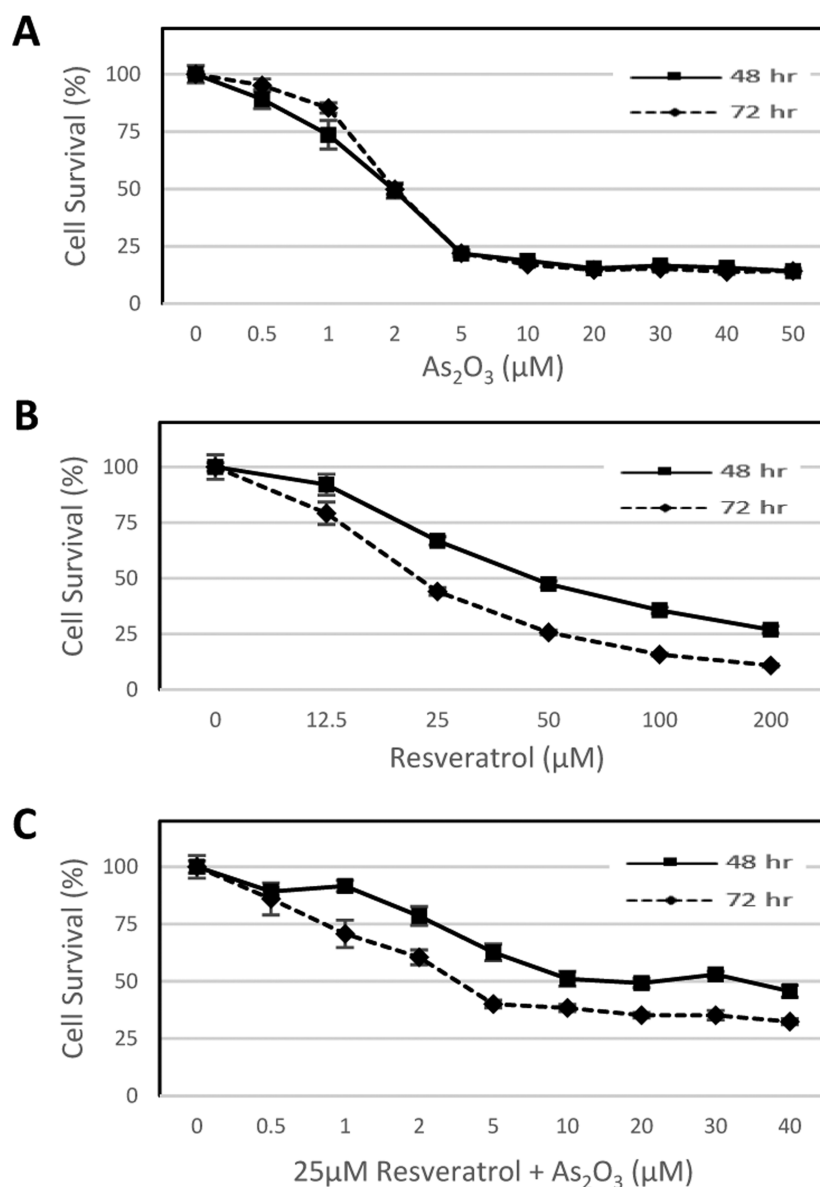


Figure 1. Viability of MCF10A cells treated with As₂O₃ with and without resveratrol. (A) Arsenic trioxide (As₂O₃) alone. (B) Resveratrol alone. (C) Arsenic trioxide (As₂O₃) + 25 μM resveratrol. Data are presented as mean ± standard error of the mean cell survival (%) from six independent experiments ($n = 6$).

groups and cancer patients (women: breast, ovarian, and thyroid; men: prostate and non-Hodgkin lymphoma), an imbalance in estrogen metabolism stems from irregular expression patterns of one or more of the four key estrogen-metabolizing enzymes: cytochrome P450 19 or aromatase, cytochrome P450 1B1 (CYP1B1), catechol-*O*-methyltransferase, and quinone reductase (NAD(P)H quinone dehydrogenase 1 (NQO1)).¹⁵ Certain naturally occurring or synthetic compounds have the ability to alter the expression of some of these enzymes; for example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) can induce CYP1B1, whereas resveratrol antagonizes this effect. At the same time, resveratrol can induce NQO1.¹⁵ Our laboratory has shown that preincubation with 25 μM resveratrol for 48 h significantly lowered the production of estrogen-DNA adduct formation in ER α -negative human breast epithelial MCF10F cells.¹⁶ Therefore, in this study, the effects of As₂O₃ on the expression levels of key estrogen-metabolizing enzymes (NQO1 and CYP1B1), and on the signaling molecule

Nrf2, were studied in the presence or absence of 25 μM resveratrol in immortalized but nontransformed ER α -negative human breast epithelial cells (MCF10A).

RESULTS AND DISCUSSION

As₂O₃ exhibited cytotoxicity at both 48 and 72 h, with an IC₅₀ at 1.45 μM for 48 h treatment (Figure 1A), which is an environmentally significant exposure. However, the presence of resveratrol, which also has some cytotoxicity (Figure 1B) at 25 μM, reduced this cytotoxicity by shifting the survival curves to the right. The IC₅₀ for the combined 48 h As₂O₃ and resveratrol treatment was 28.69 μM. The rightward shift in the survival curve was lesser for 72 h treatment compared to that for 48 h treatment (Figure 1C). This indicates that the rescue effect from resveratrol is more pronounced at 48 h.

As₂O₃, with or without 25 μM resveratrol, induced the enzyme NQO1 in a dose-dependent manner, but no additive or synergistic effects were observed (Figure 2A,B). Resveratrol has

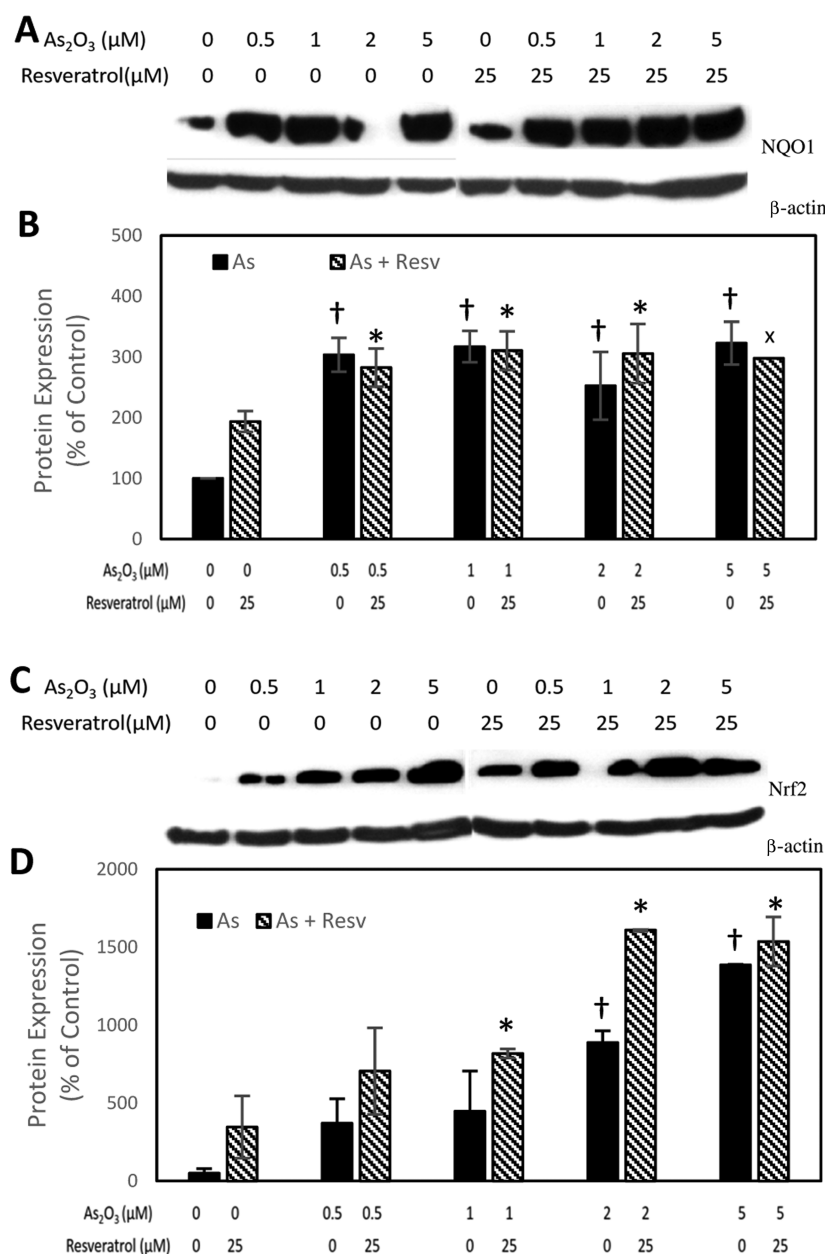


Figure 2. Induction of NQO1 (A, B) and Nrf2 (C, D) by As₂O₃ with and without resveratrol. (A, C) Representative images. (B, D) Quantitative data. Data are presented as mean \pm standard error of the mean protein expression (%) from three independent experiments ($n = 3$), X = mean of two experiments. A † indicates significant difference ($p < 0.05$) between As₂O₃ treatment groups and control, whereas an * indicates significant difference ($p < 0.05$) between As₂O₃ treatment groups and control in the resveratrol group.

been shown to act via the signaling molecule Nrf2, and NQO1 has been implicated as a downstream gene of Nrf2.¹⁷ This prompted us to study the effect of As₂O₃ on the expression level of Nrf2. As₂O₃, in both the presence and absence of 25 μM resveratrol, induced Nrf2 in a dose-dependent fashion, but again no additive or synergistic effects were observed (Figure 2C,D). Our finding is in accordance with a study in mouse hepatoma cell line hep1c1c7, in which arsenic was shown to induce Nrf2 protein in a dose-dependent fashion, leading to a robust induction of NQO1.¹⁸ Furthermore, in MCF10A cells the extent of induction was much greater for Nrf2 compared to that for NQO1, but this may stem from the fact that resting cells have a very low-level basal expression of Nrf2 ($t_{1/2} = 10\text{--}20$ min).¹⁹ Similarly, disproportionate induction of NQO1 and Nrf2 by As₂O₃ has been reported in male Kunming mouse

testis in both the presence and absence of antioxidants such as lutein.²⁰

CYP1B1 is another key estrogen-metabolizing enzyme that is overexpressed in a variety of cancers, including breast cancer, and is subject to regulation through both hormonal and a putative aryl hydrocarbon receptor (AhR) pathways; the latter can be modulated by resveratrol.²¹ Previous studies on the male C57Bl/6 mouse heart have reported that 12.5 mg/kg As(III) induces CYP1B1 mRNA levels by 150%.²² We observed, however, that As₂O₃ treatment (0.5–5 μM) of human breast epithelial MCF10A cells caused a repression of CYP1B1 protein in a dose-dependent manner. Resveratrol was earlier shown to downregulate TCDD-induced CYP1B1 expression in MCF10F cells.²³ Interestingly, the addition of 25 μM resveratrol rescued As₂O₃-suppressed CYP1B1 expression and

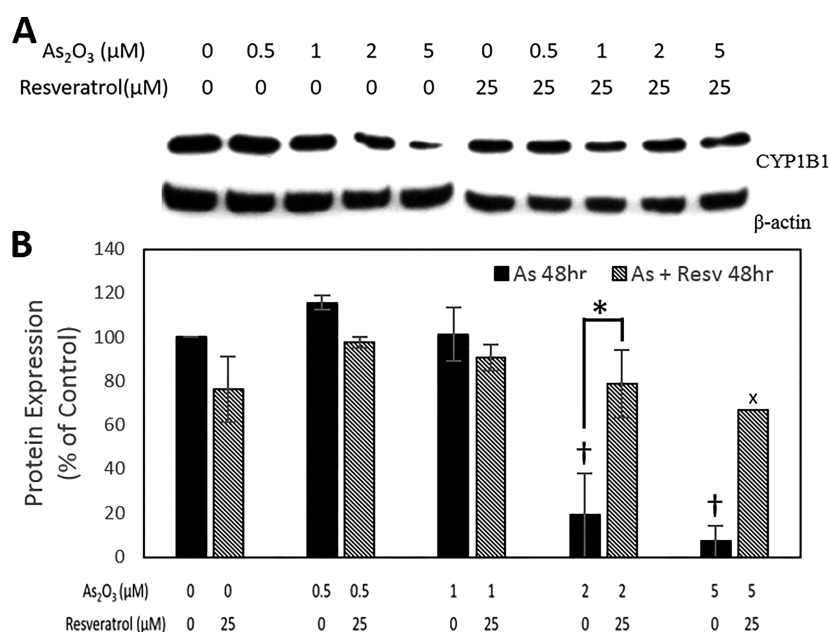


Figure 3. Repression of CYP1B1 by As₂O₃ with and without resveratrol. (A) Representative image. (B) Quantitative data. Data are presented as mean \pm standard error of the mean protein expression (%) from three independent experiments ($n = 3$), X = mean of two experiments. A † indicates significant difference ($p < 0.05$) between As₂O₃ treatment groups and control, whereas an * indicates significant difference ($p < 0.05$) between one particular As₂O₃ treatment with the inclusion or exclusion of resveratrol.

kept it at a nearly constant level (Figure 3A,B). To confirm this interesting observation, the expression of CYP1B1 enzyme was further studied simultaneously in three different nontransformed cell lines derived from human breast epithelia, namely MCF10A, MCF10F, and MCF12F cells. In this study, the cells were also treated with a higher dose of 7.5 μ M As₂O₃. The repressive effect of As₂O₃ on CYP1B1 expression was less pronounced in MCF10F cells, leaving little room for resveratrol to exert any rescue effect (Supporting Information, Figure S1B). Furthermore, such repressive effect of As₂O₃ on CYP1B1 expression was altogether absent in MCF12F cells (Supporting Information, Figure S1C). Collectively, these results suggest different degrees of modulatory effects of As₂O₃. One possibility is its differential cytotoxicity, as we have observed between MCF10A and MCF12F cells (IC₅₀ value for MCF10A is 1.45 μ M for 48 h treatment, whereas the same for MCF12F is 11.65 μ M; Figure 1A vs Supporting Information Figure S2). As₂O₃ is not considered a ligand for AhR; therefore, the observed effects of As₂O₃ might be mediated through a hormonal pathway.

Conclusions. In the present study involving nontransformed breast epithelial MCF10A cells, 25 μ M resveratrol was shown to ameliorate As₂O₃-induced cytotoxicity, with the beneficial effect being more prominent after 48 h treatment. As expected, both As₂O₃ and resveratrol induced the expression of the catechol quinone-quenching enzyme NQO1, possibly via the induction of the signaling protein Nrf2. To the best of our knowledge, however, this is the first time resveratrol has been shown to rescue As₂O₃-suppressed CYP1B1 expression back to the baseline levels. Collectively, these results suggest that 25 μ M resveratrol has the ability to modulate the effects of As₂O₃ on the expression of estrogen-metabolizing enzymes in MCF10A cells. Whether such modulation leads to any potential benefit in estrogen metabolism and carcinogenesis needs to be addressed in future studies of the effects of As₂O₃ and resveratrol on estrogen metabolism.

EXPERIMENTAL PROCEDURES

Cytotoxicity. Cells were seeded in a 96-well plate (seeding density: MCF10A 3000 cells per well, MCF12F 5000 cells per well) in estrogen- and phenol-red-indicator-free media and treated with 0.5–50 μ M total concentration of As₂O₃ (purity > 99.5%; Sigma-Aldrich, St. Louis, MO), 12.5–200 μ M total concentration of resveratrol (purity \geq 98%; Cayman Chemical, Ann Arbor, MI), or 0.5–50 μ M total concentration of As₂O₃ + 25 μ M resveratrol for two time points (48 and 72 h). Finally, MTT (Calbiochem, San Diego, CA) was used to assess the cell viability.

Protein Expression Studies. Expression levels of the key estrogen-metabolizing enzymes NQO1 and CYP1B1 and of cell signaling protein Nrf2 were studied under different treatment regimens. Cells were seeded in a 6-well plate (seeding density: MCF10A and MCF10F 0.8×10^6 cells per well, MCF12F 1×10^6 cells per well) in estrogen- and phenol-red-indicator-free media and treated with 0.5–5 μ M total concentration of As₂O₃ \pm 25 μ M resveratrol for 48 h. Following cell lysis with radioimmunoprecipitation assay buffer containing protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland), total protein was estimated by the Bradford assay, and 10–30 μ g of total protein was resolved on a 15% polyacrylamide gel and transferred to poly(vinylidene difluoride) membranes. The membranes were probed with anti-NQO1, anti-Nrf2 (Abcam, Cambridge, MA), anti-CYP1B1 (LSBio, Seattle, WA), or anti- β -actin (Santa Cruz, Dallas, TX) primary antibodies overnight at 4 $^{\circ}$ C and secondary antibodies for 1 h at room temperature. Detection was performed with ECL Western Blotting Detection Reagents (GE Healthcare/Amersham, Little Chalfont, U.K.). Both the treatment and western blotting were performed three times to achieve statistical significance.

Statistical Analysis. To determine whether the differences observed were statistically significant, a 2-sample t -test was performed. α was set at 0.05 for all statistical tests, and data with $p < 0.05$ were considered to be significantly different.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsomega.7b01727](https://doi.org/10.1021/acsomega.7b01727).

The expression of CYP1B1 by As₂O₃ with and without resveratrol in MCF10A, MCF10F, and MCF12F cells; the viability of MCF12F cells treated with As₂O₃ (PDF)

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Author Contributions

The manuscript was written through contributions of all authors.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

As₂O₃, arsenic trioxide; NQO1, NAD(P)H quinone dehydrogenase 1; Nrf2, NFE2-related factor 2; CYP1B1, cytochrome P450 1B1; CYP19, cytochrome P450 19; COMT, catechol-O-methyltransferase; E₂, estradiol; 4-OHE₂, 4-hydroxyestradiol; E₂-3,4-Q, estradiol-3,4-quinone; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin

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